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"Programmable transcription of MECP2 in patient iPSC-derived neurons using CRISPR/dCas9 as a putative therapeutic for Rett Syndrome"

Neurological diseases are a heterogeneous group of disorders caused by alterations in nervous system function and due to technological advances over the last decade; many of these disorders can be attributed to genetic factors such as chromosomal aberrations or gene mutations. Rett syndrome (RTT) is a severe neurodevelopmental disorder characterized by a period of normal infancy followed by regression in developmental milestones, resulting in mental retardation with absence of speech, ataxia, loss of purposeful hand movements, and seizures. RTT is caused by mutations in MECP2 on the X chromosome and shows an X-linked dominant inheritance pattern, exclusively in females. Because of X chromosome inactivation (XCI), females with RTT are mosaic of cells expressing the mutant and wild-type alleles of MECP2. The precise function of MeCP2 remains unclear, but it appears to play a role in synapse formation. Although MeCP2 was predicted to be a global transcriptional repressor of methylated genes, the roles have since expanded to include structural heterochromatin, chromatin looping, alternative splicing, activation-dependent transcriptional regulation, long-range modulation of genes, and even direct promoter activation. MeCP2 is only required during postnatal brain maturational development, when expression is elevated.

Our research platform involves creating gene-modifying proteins that can recognize and bind to specific gene sequences in the MeCP2 gene. The CRISPR system can be paired with a variety of other effector domains to turn on or enhance gene expression. However, regions of the genome that are amenable to regulating gene expression are poorly understood. Our preliminary results demonstrate that MeCP2 can be efficiently targeted and enhanced following transfection of a pool of gRNA with a transcriptional activation domain. This research proposal will identify cis regulatory regions proximal and distal to the MECP2 start site that are amenable to targeted gene activation and durable chromatin remodeling. We also propose to test these novel identified in a panel of human fibroblasts harboring naturally occurring MECP2 mutations to demonstrate reactivation following transduction of our therapeutic CRISPR/dCas9 therapeutic. We also propose to evaluate this approach in patient iPSC-derived neurons to demonstrate rescue of gene and morphological abnormalities. This platform may hold great promise in the future for those suffering from genetic linked disorders. If our therapeutic product and delivery platform proves to be effective we would be able to accelerate our research through the FDA pipeline.